AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Currently Amended): A method for obtaining a stable pluripotent human blastocyst-derived stem cell line comprising:

i) using a fertilized human oocyte of optionally grade 1 or 2 to obtain a human blastocyst of optionally grade A or B;

ii) co-culturing the human blastocyst with fibroblast feeder cells to establish one or more colonies of inner cell mass cells;

iii) isolating the inner cell mass cells by mechanical dissection into pieces, thereby isolating the inner cell mass cells without the use of immunosurgery;

iv) co-culturing the inner cell mass cells with fibroblast feeder cells to obtain human blastocyst-derived stem cell colonies; and

v) propagating the human blastocyst-derived stem cell colonies by repeatedly passaging the human blastocyst-derived stem cell colonies every 4 to 5 days, wherein each passaging step comprises manually dissecting the inner homogeneous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells; thereby obtaining a stable pluripotent human blastocyst-derived stem cell line;

~~wherein the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery~~

wherein the human blastocyst obtained in i) is a hatched human blastocyst.

Claim 2 (Currently Amended): A method for obtaining a stable pluripotent human blastocyst-derived stem cell line comprising:

i) using a fertilized human oocyte of grade 1 or 2 to obtain a human blastocyst;

ii) co-culturing the human blastocyst with fibroblast feeder cells to establish one or more colonies of inner cell mass cells;

iii) isolating the inner cell mass cells by mechanical dissection into pieces, thereby isolating the inner cell mass cells without the use of immunosurgery;

iv) co-culturing the inner cell mass cells with fibroblast feeder cells to obtain human blastocyst-derived stem cell colonies; and

v) propagating the human blastocyst-derived stem cell colonies by repeatedly passaging the human blastocyst-derived stem cell colonies every 4 to 5 days, wherein each passaging step comprises manually dissecting the inner homogeneous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells; thereby obtaining a stable pluripotent human blastocyst-derived stem cell line;

~~wherein the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery~~

wherein the human blastocyst obtained in i) is a hatched human blastocyst.

Claim 3 (Currently Amended): A method for obtaining a stable pluripotent human blastocyst-derived stem cell line comprising:

i) using a fertilized human oocyte to obtain a human blastocyst of grade A or B;

ii) co-culturing the human blastocyst with fibroblast feeder cells to establish one or more colonies of inner cell mass cells;

iii) isolating the inner cell mass cells by mechanical dissection into pieces,

thereby isolating the inner cell mass cells without the use of immunosurgery;

iv) co-culturing the inner cell mass cells with fibroblast feeder cells to obtain human blastocyst-derived stem cell colonies; and

v) propagating the human blastocyst-derived stem cell colonies by repeatedly passaging the human blastocyst-derived stem cell colonies every 4 to 5 days, wherein each passaging step comprises manually dissecting the inner homogeneous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells; thereby obtaining a stable pluripotent human blastocyst-derived stem cell line;

~~wherein the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery~~

wherein the human blastocyst obtained in i) is a hatched human blastocyst.

Claim 4 (Currently Amended): A method for obtaining a stable pluripotent human blastocyst-derived stem cell line comprising:

i) using a fertilized human oocyte optionally of grade 1 or 2 to obtain a human blastocyst of optionally grade A or B;

ii) co-culturing the human blastocyst with fibroblast feeder cells to establish one or more colonies of inner cell mass cells[[,]] ;

iii) isolating the inner cell mass cells by mechanical dissection into pieces, thereby isolating the inner cell mass cells without the use of immunosurgery;

iv) co-culturing the inner cell mass cells with fibroblast feeder cells to obtain human blastocyst-derived stem cell colonies; and

v) propagating the human blastocyst-derived stem cell colonies by repeatedly passaging the human blastocyst-derived stem cell colonies every 4 to 5 days, wherein each

passaging step comprises manually dissecting the inner homogeneous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells of a density of less than 60,000 cells per cm^2; thereby obtaining a stable pluripotent human blastocyst-derived stem cell line;

~~wherein the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery~~

wherein the human blastocyst obtained in i) is a hatched human blastocyst.

Claim 5 (Previously Presented): The method of claim 1, wherein the blastocyst in step i) is a spontaneously hatched blastocyst.

Claims 6-8 (Canceled).

Claim 9 (Previously Presented): The method of claim 1, wherein propagating the pluripotent human blastocyst-derived stem cell line comprises culturing the pluripotent human blastocyst-derived stem cell line with fibroblast feeder cells of a density of less than 60,000 cells per cm^2.

Claim 10 (Previously Presented): The method of claim 9, wherein propagating the blastocyst-derived stem cell line comprises culturing the pluripotent human blastocyst-derived stem cell line with fibroblast feeder cells of a density of about 45,000 cells per cm^2.

Claim 11 (Previously Presented): The method of claim 1, wherein the propagation of the pluripotent human blastocyst-derived stem cell line in step iv) comprises passage of the fibroblast feeder cells at the most 3 times.

Claim 12 (Previously Presented): The method of claim 1, wherein the zona pellucida of the blastocyst has been at least partially digested prior to step ii).

Claim 13 (Previously Presented): The method of claim 12, wherein the zona pellucida of the blastocyst has been at least partially digested with a digestive agent selected from the group consisting of acidic reacting substances, enzymes and mixtures thereof.

Claim 14 (Previously Presented): The method of claim 1, wherein at least one of step ii) and step iv) is performed in an agent that improves at least one of (a) the attachment of the blastocysts to the fibroblast feeder cells and (b) the attachment of the inner cell mass cells to the fibroblast feeder cells.

Claim 15 (Previously Presented): The method of claim 14, wherein the agent is a hyaluronic acid.

Claim 16 (Previously Presented): The method of claim 1, wherein the fibroblast feeder cells in at least one of step ii) and step iv) are embryonic fibroblast feeder cells.

Claim 17 (Previously Presented): The method of claim 1, wherein the fibroblast feeder cells employed in steps ii) and iv) are the same or different and the fibroblast feeder cells originate from an animal source.

Claim 18 (Previously Presented): The method of claim 17, wherein the fibroblast feeder cells of at least one of step ii) and step iv) are of mouse or human origin.

Claim 19 (Previously Presented): The method of claim 1, wherein the fibroblast feeder cells of at least one of step ii) and step iv) are mitotically inactivated.

Claim 20 (Previously Presented): The method of claim 1, wherein the pluripotent human blastocyst-derived stem cell line

i) exhibits proliferation capacity in an undifferentiated state for more than 21 months when grown on mitotically inactivated embryonic fibroblast feeder cells;

ii) exhibits normal euploid chromosomal karyotype;

iii) maintains potential to develop into derivatives of all types of germ layers both *in vitro* and *in vivo*;

iv) exhibits at least two of the group of molecular markers consisting of OCT-4, alkaline phosphatase, SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, and the protein core of a keratin sulfate/chondroitin sulfate pericellular matrix proteinglycan recognized by the monoclonal antibody GCTM-2;

v) does not exhibit molecular marker SSEA-1 or other differentiation markers;

vi) retains pluripotency and forms teratomas in vivo when injected into immuno-compromised mice; and

vii) is capable of differentiation.

Claim 21 (Canceled).

Claim 22 (Withdrawn): The method of claim 1, wherein the stem cell line has the ability of differentiating into an insulin producing cells.

Claim 23 (Withdrawn): The method of claim 22, wherein the insulin producing cells form islet-like structures.

Claim 24 (Withdrawn): The method of claim 22, wherein the amount of insulin producing β-cells which is derived from the pluripotent human BS cell line is higher than 25%.

Claim 25 (Withdrawn): The method of claim 22, wherein the insulin producing cell line produces at least about 300 ng insulin/mg total protein.

Claim 26 (Withdrawn): The method of claim 1, wherein the blastocyst-derived stem cells have the ability to differentiate into differentiated cells, which display the expression of pancreatic cell type markers, including at least one of a group consisting of insulin, Glut-2, Pdx-1, glucokinase, glucagons, and somatostatin.

Claim 27 (Withdrawn): The method of claim 1, wherein the blastocyst-derived stem cells have the ability to differentiate into insulin-producing cells that organize into islet-like structures comprising an inner core of β-cells surrounded by an outer layer of neuron-type cells, which neuron-type cells display expression of at least one of the following neuronal cell type markers, including neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase, and Map 2.

Claim 28 (Withdrawn): The method of claim 1, wherein the blastocyst-derived stem cells are capable of differentiated into cells, which express at least one neuronal cell type

markers selected from the group consisting of neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase, and Map 2.

Claim 29 (Withdrawn): A preparation of differentiated cells derived from the blastocyst-derived stem cells obtained by the method of claim 1 for preventing or treating pathologies or diseases caused by tissue degeneration.

Claim 30 (Withdrawn): A preparation of differentiated cells derived from the blastocyst-derived stem cells obtained by the method of claim 1 for preventing or treating pathologies or diseases in the pancreas.

Claim 31 (Withdrawn): The preparation of differentiated cells of claim 30, wherein the disease is diabetes.

Claim 32 (Withdrawn): The preparation of differentiated cells of claim 28, wherein the disease is type 1 diabetes.

Claim 33 (Withdrawn): A preparation of differentiated cells derived from the blastocyst-derived stem cell line obtained by the method of claim 1 for preventing or treating pathologies or diseases in the nervous system.

Claim 34 (Withdrawn): The preparation of differentiated cells of claim 33, in which the disease is selected from the group consisting of multiple sclerosis, spinal chord injury, an encephalopathy, Parkinson's disease, Huntingdon's disease, stroke, a traumatic brain injury, a hypoxia induced brain injury, an ischemia induced brain injury, a hypoglycemic brain injury,

a degenerative disorder of the nervous system, a brain tumor, and a neuropathy in the peripheral nervous system.

Claim 35 (Previously Presented): A kit for performing the method of claim 1, comprising human blastocysts with an intact zona pellucida or spontaneously hatched blastocysts, and at least two of the following components in separate compartments: hyaluronic acid, pronase, BS-cell medium, and human or mouse embryonic fibroblast feeder cells.

Claim 36 (Withdrawn): A method for producing an essentially pure preparation of insulin-producing differentiated stem cells, comprising:

i) expanding human blastocyst-derived stem cells by growing the blastocyst-derived stem cells on an inactivated feeder cell layer in a suitable medium;

ii) generating blastocyst-derived stem cell bodies by dissociating colonies formed in step i) into smaller aggregates or individual cells, followed by transferring said aggregates or individual cells in to non-adherent containers wherein said aggregate or individual cells are incubated in a suitable medium;

iii) plating the blastocyst-derived stem cell bodies in containers in a suitable medium;

iv) selecting nestin-positive neural precursors in ITFSn medium;

v) expanding pancreatic endocrine progenitor cells in N2-medium comprising B27 media complement and basic fibroblast growth factor; and

vi) changing the medium to a basic fibroblast growth factor-free N2 medium.

Claim 37 (Withdrawn): The method of claim 36, wherein the human blastocyst-derived stem cells are obtained by:

i) using a fertilized oocyte of grade 1 or 2 to obtain a blastocyst of grade A or B;

ii) co-culturing the blastocyst with feeder cells to establish one or more colonies of inner cell mass cells;

iii) isolating the inner cell mass cells by mechanical dissection; and

iv) co-culturing the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line.

Claim 38 (Withdrawn): The method of claim 36, wherein the medium used in step i) is human blastocyst-derived stem cell medium.

Claim 39 (Withdrawn): The method of claim 36, wherein the medium used in step ii) is blastocyst-derived stem cell body medium.

Claim 40 (Withdrawn): The method of claim 36, wherein the medium used in step iii) is blastocyst-derived stem cell body medium.

Claim 41 (Withdrawn): The method of claim 36, wherein nicotinamide is added after step vi).

Claim 42 (Withdrawn): An essentially pure preparation of differentiated stem cells, wherein said stem cells display an expression of pancreatic cell type markers wherein said marker is at least one or more of insulin, Glut-2, Pdx-1, glucokinase, glucagons, or somatostatin.

Claim 43 (Withdrawn): The preparation of claim 42, which is capable of producing at least about 320 ng insulin/mg total protein.

Claim 44 (Withdrawn): The preparation of claim 42, wherein the preparation comprises at least 25% insulin producing cells.

Claim 45 (Withdrawn): The preparation of claim 42, wherein said stem cells are organized into islet-like structures comprising an inner core of β-cells surrounded by an outer layer of neuron-type cells, wherein the neuron-type cells express at least one of the neuronal cell type markers selected from the group consisting of: neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase, and Map 2.

Claim 46 (Withdrawn-Currently Amended): The preparation of claim 42, obtained by:

i) expanding human blastocyst-derived stem cells by growing the blastocyst-derived stem cells on an inactivated feeder cell layer in a suitable medium;

ii) generating blastocyst-derived stem cell bodies by dissociating colonies formed in step i) into smaller aggregates or individual cells, followed by transferring said aggregates or individual cells in to non-adherent containers wherein said aggregate or individual cells are incubated in a suitable medium; ~~and~~

iii) plating the blastocyst-derived stem cell bodies in containers in a suitable medium;

iv) selecting nestin-positive neural precursors in ITFSn medium;

v) expanding pancreatic endocrine progenitor cells in N2-medium comprising B27 media complement and basic fibroblast growth factor; and

vi) changing the medium to a basic fibroblast growth factor-free N2 medium.

Claim 47 (Withdrawn): An essentially pure preparation of differentiated stem cells, wherein the stem cells express at least one of the neuronal cell type markers selected from the group consisting of: neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase, or Map 2.

Claim 48 (Withdrawn-Currently Amended): The preparation of claim 47 obtained by:

i) expanding human blastocyst-derived stem cells by growing the blastocyst-derived stem cells on an inactivated feeder cell layer in a suitable medium;

ii) generating blastocyst-derived stem cell bodies by dissociating colonies formed in step i) into smaller aggregates or individual cells, followed by transferring said aggregates or individual cells in to non-adherent containers wherein said aggregate or individual cells are incubated in a suitable medium; ~~and~~

iii) plating the blastocyst-derived stem cell bodies in containers in a suitable medium;

iv) selecting nestin-positive neural precursors in ITFSn medium;

v) expanding pancreatic endocrine progenitor cells in N2-medium comprising B27 media complement and basic fibroblast growth factor; and

vi) changing the medium to a basic fibroblast growth factor-free N2 medium.

Claim 49 (Withdrawn-Currently Amended): An essentially pure preparation of stem cells obtained by:

i) expanding human blastocyst-derived stem cells by growing the blastocyst-derived stem cells on an inactivated feeder cell layer in a suitable medium;

ii) generating blastocyst-derived stem cell bodies by dissociating colonies formed in step i) into smaller aggregates or individual cells, followed by transferring said aggregates or individual cells in to non-adherent containers wherein said aggregate or individual cells are incubated in a suitable medium; ~~and~~

iii) plating the blastocyst-derived stem cell bodies in containers in a suitable medium;

iv) selecting nestin-positive neural precursors in ITFSn medium;

v) expanding pancreatic endocrine progenitor cells in N2-medium comprising B27 media complement and basic fibroblast growth factor; and

vi) changing the medium to a basic fibroblast growth factor-free N2 medium.

Claim 50 (Withdrawn): An essentially pure preparation of differentiated stem cells of claim 42 for preventing or treating pathologies or diseases in the pancreas.

Claim 51 (Withdrawn): The preparation of claim 50, wherein the disease is diabetes.

Claim 52 (Withdrawn): The preparation of claim 50, wherein in which the disease is type 1 diabetes.

Claim 53 (Withdrawn): The preparation of claim 47 for treating pathologies or diseases in the nervous system.

Claim 54 (Withdrawn): The preparation of claim 53, wherein the disease is selected from the group consisting of multiple sclerosis, spinal chord injury, an encephalopathy, Parkinson's disease, Huntingdon's disease, stroke, a traumatic brain injury, a hypoxia induced brain injury, an ischemia induced brain injury, a hypoglycemic brain injury, a

degenerative disorder of the nervous system, a brain tumor, and a neuropathy in the peripheral nervous system.

Claim 55 (Withdrawn): A kit for performing the method of claim 36 comprising at least two of the following components in separate compartments: mitomycin C, hBS medium, BS cell body medium, ITSFn-medium, N2-medium, B27-media supplement, nicotinamide, and bFGF.

Claim 56 (Withdrawn): The kit of claim 55, further comprising an essentially pure human blastocyst-derived stem cell line obtained by:

i) using a fertilized oocyte of grade 1 or 2 to obtain a blastocyst of grade A or B;

ii) co-culturing the blastocyst with feeder cells to establish one or more colonies of inner cell mass cells;

iii) isolating the inner cell mass cells by mechanical dissection; and

iv) co-culturing the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line.

Claims 57-59 (Canceled).

Claim 60 (Previously Presented): The method of claim 9, wherein the fibroblast feeder cells are at a density of less than 55,000 cells per cm^2.

Claim 61 (Previously Presented): The method of claim 9, wherein the fibroblast feeder cells are at a density of less than 50,000 cells per cm^2.

Claim 62 (Previously Presented): The method of claim 1, wherein the fibroblast feeder cells of step ii) and step iv) are human or mouse embryonic fibroblast feeder cells.

Claim 63 (Canceled).

Claim 64 (Previously Presented): The method of claim 62, wherein the feeder cells of step ii) and step iv) are human embryonic fibroblasts.

Claim 65 (Previously Presented): The method of claim 62, wherein the feeder cells of step ii) and step iv) are mouse embryonic fibroblasts.